



Effects of digital phase-conjugate light intensity on time-reversal imaging through animal tissue

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Abstract: For transillumination imaging of animal tissues, we have attempted to suppress the scattering effect in a turbid medium using the time-reversal principle of phase-conjugate light. We constructed a digital phase-conjugate system to enable intensity modulation and phase modulation. Using this system, we clarified the effectiveness of the intensity information for restoration of the original light distribution through a turbid medium. By varying the scattering coefficient of the medium, we clarified the limit of time-reversal ability with intensity information of the phase-conjugate light. Experiment results demonstrated the applicability of the proposed technique to animal tissue.

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1. Introduction

The use of light in many biomedical applications has progressed rapidly recently. Benefits of using light include its non-invasiveness to animal tissue and its capability of functional imaging. However, for bioimaging and measurements using light, the strong scattering property of animal tissue represents an important hindrance. Therefore, some technique to suppress scattering effects is necessary for practical applications of light in these fields. In recent years, a principle of scattering suppression using the time-reverse capability of phase-conjugate light has been proposed [1–16]. Various attempts based on this principle have been undertaken, such as the time-reversed ultrasonically encoded (TRUE) method [1,4–8]. Many of these methods are aimed at focusing the light in a turbid medium. That practice tends to produce complex techniques to generate the phase-conjugate light. Moreover, the analysis of the phase-conjugate light separated from other effects becomes more difficult. Therefore, to enhance biomedical applications, we investigated the scattering suppression effects of phase-conjugate light with a non-focusing system.

To generate the phase-conjugate light of a certain optical signal, the signal light and the reference light are first made to interfere mutually. Then the phase information of the optical signal is measured. Next, the reference light is irradiated to the spatial light modulator (SLM) and is spatially modulated with the phase-conjugate information. In conventional bioimaging using digital phase-conjugate system [1,2,7,8,16], phase-conjugate light is generated with a single SLM and CMOS camera [17,18]. Usually in such a system, phase modulation is emphasized. Intensity modulation is not an objective. Therefore, the intensity information of the generated phase-conjugate light results in the intensity distribution of the reference light irradiated to the SLM: if the spatial intensity distribution of the reference light is a plane wave or Gaussian distribution, the phase-conjugate light also becomes the same plane wave or Gaussian distribution. Often, a uniform distribution has been assumed for the application of the digital phase-conjugate light [19]. However, in the case of a heterogeneous animal sample or when the incident light shape has a distribution, the intensity distribution of the signal light becomes spatially non-uniform to a marked degree. To fulfill the phase-conjugate light potential, the light-phase must not only be inverted; the intensity distribution must also be the

same as that of the signal light. To overcome this difficulty, we constructed a digital phase-conjugate system to enable phase and intensity modulation (PIM).

If correct digital phase-conjugate light is obtainable, then the scattering effect in transillumination imaging can be suppressed effectively by the time-reversal principle. For this study, we investigated the influence of the intensity component of digital phase-conjugate light on suppression of the scattering effect in transillumination imaging, and verified its importance. We also strove to clarify the limit of the scattering suppression capability of this technique with a non-focusing system.

2. Digital phase-conjugate system with intensity modulation

Conventional biological imaging systems with digital phase-conjugate light are generally composed of one SLM and CMOS camera (Fig. 1(a)) [1,2,7,8,16]. In such systems, holograms of reference light and signal light are first recorded by the CMOS camera. After the phase information of the signal light is obtained from the hologram, the phase pattern of the conjugate to the obtained phase information is calculated. The SLM is irradiated with the same reference light as that used for the phase information measurement. The phase-conjugate light is generated by modulating the reference light with the calculated phase pattern. The phase-conjugate light is generated by modulating the reference light with the calculated phase pattern. In this way, the spatial intensity distribution of the generated phase-conjugate light is the same as that of the reference light with no intensity information of the original signal light.

We can expect a scattering suppression effect by the backpropagation of this phase-conjugate light. However, in the technique with a single SLM described above, the spatial intensity distribution of the backpropagated light is dominated by that of the reference light such as the plane or the Gaussian distributions. In transillumination imaging applications, the quality of the intensity distribution is of prime importance. Therefore, we can expect to achieve marked improvement in scattering suppression performance by using backpropagated light that is matched not only in phase but also in intensity. For this study, to assess the influence of the intensity component of the phase-conjugate light, we applied spatial intensity modulation to the reference light. Therefore, as presented in Fig. 1(b), a transmitting SLM and a polarizer were arranged on the reference light side. Thereby, the intensity modulation is enabled by spatially modulating the reference light with the transmitting SLM, with passage through the polarizer. Okamoto et al. [20] also proposed a similar principle of the simultaneous intensity and phase modulation in optics study. There, reflecting SLM of two types are used. Intensity modulation and phase modulation are performed simultaneously by overlapping the modulated light. By contrast, with our system, we used a transmission type SLM for intensity modulation. It has greater intensity loss of the generated phase-conjugate light than their system with a reflecting type SLM. However, alignment between two SLMs in our system is not as complicated as that in the system with two reflective SLMs because we can observe the modulation pattern of the intensity through the transmission type SLM directly using the CMOS camera. Therefore, in this study, the configuration of Fig. 1(b) was adopted, which enables direct comparison with the conventional method [17,18].

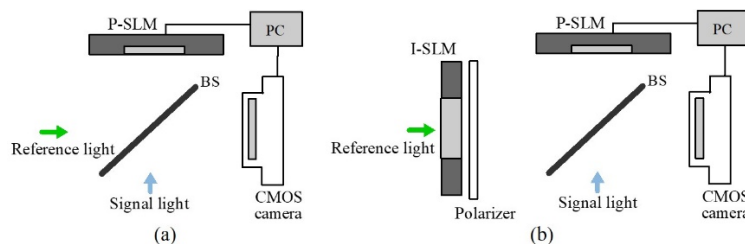


Fig. 1. Digital phase-conjugate light generation system: (a) phase-only modulation and (b) phase and intensity modulation.

The principle of generating phase-conjugate light with intensity modulation constructed in the present study is presented in Fig. 2. Using this system, three processes are necessary to generate phase-conjugate light. In the first step, we observe only the signal light with the CMOS camera and obtain the spatial intensity distribution of the signal light (Fig. 2(a)). Based on the obtained intensity distribution, the reference light intensity is modulated by the transmitting SLM (I-SLM), thereby making the intensity distribution of the reference light equal to that of the signal light. In the second step, this reference beam and signal light are made to interfere mutually. The hologram is recorded using a CMOS camera (Fig. 2(b)). In hologram imaging, the reference light phase is shifted in increments of $\pi/2$ rad to record four holograms. Based on these four holograms, a spatial phase distribution of the signal light is calculated using a four-step phase shift method [21]. Then, the phase pattern which is conjugate to the obtained phase distribution is calculated. In the third step, the intensity-modulated reference light reflected by the beam splitter is phase-modulated and reflected by the reflecting SLM (P-SLM) based on the conjugate phase pattern. Consequently, the reflected light has the same distributions of intensity and conjugate phase as the original signal light has. It propagates back in the direction opposite to the signal light to achieve the time-reversal principle.

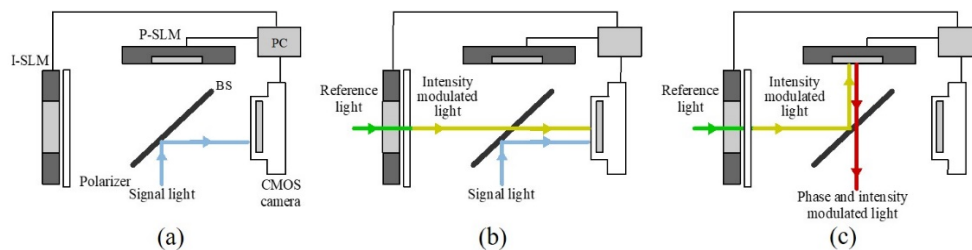


Fig. 2. Principle of phase-conjugate light generation with intensity modulation: (a) intensity recording, (b) phase recording, (c) generation of intensity modulated phase-conjugate light.

3. Experimental system

In experiments, we investigated the influence of the intensity information of the phase-conjugate light on suppression of the scattering effect in optical transillumination imaging. Phase-conjugate light was generated from the scattered light after passing through the scattering medium. Then restoration of the incident light shape before scattering was attempted. The experimental system is depicted in Fig. 3. To confirm the effect of the time-reversal function of the phase-conjugate light, we tried to make the experimental system as simple as possible, excluding elements such as those for the confocal system [3].

The part surrounded by the red dashed line in Fig. 3(a) was constructed for the digital phase-conjugate. As a light source, a diode pumped solid state (DPSS) laser (Ventus 532; Laser Quantum) with 532 nm wavelength was used. Light emitted from the light source was divided into the reference light side and the signal light side by the polarization beam splitter (PBS). The system was made up with SLMs for intensity (I-SLM, LC2012, 1024×768 pixels, pixel size $36 \times 36 \mu\text{m}$; Holoeye Photonics AG), and for phase (P-SLM, X10468-01, 792×600 pixels, pixel size $20 \times 20 \mu\text{m}$; Hamamatsu Photonics KK), and a CMOS camera (CMOS camera 1, Zyla 5.5, 2560×2160 pixels, pixel size $6.5 \times 6.5 \mu\text{m}$; Andor Technology) for intensity and phase measurements. In the optical system for digital phase-conjugate, it is necessary to match the pixels of the P-SLM and the CMOS camera on a one-to-one basis. Therefore, pixel matching of P-SLM and CMOS camera 1 was realized by placing a plano-convex lens (L3, 100 mm) in front of the CMOS camera 1 and focusing the light. Regarding the I-SLM, we matched it with the P-SLM and the CMOS camera 1 using only a 439×333 subset of the pixels. Results show that the spatial resolution of the intensity distribution of the

generated phase-conjugate light was $36 \times 36 \mu\text{m}$. The spatial resolution of the phase distribution was $20 \times 20 \mu\text{m}$.

Figure 3(a) portrays the experimental system when intensity information was acquired. Uniform light was irradiated to the object pattern (USAF 1951 target; Edmund). The light transmitted through the scattering medium was used as the signal light. After passing through the scatterer, the signal light is guided to the digital phase-conjugate light system. Its intensity distribution is recorded using CMOS camera 1.

Figure 3(b) depicts the experimental system when phase information is acquired. With the intensity distribution of the signal light recorded in the previous process, the reference light intensity is modulated by the I-SLM. As a result, the intensity distribution of the reference light becomes equal to the signal light. A hologram generated by interference between the reference light and the signal light is recorded by CMOS camera 1. The hologram was recorded while shifting the optical path of the reference light by $1/4$ wavelength. A piezo stage (SFS - H 40 X; Sigma Koki Co. Ltd.) was used to shift the optical path. From these holograms, the spatial phase distribution of the signal light was calculated using the four-step phase shift method [21]. The surface of each SLM has anomalous curvature. For that reason, the modulation pattern was corrected using the calibration distribution obtained before the measurement.

Figure 3(c) portrays the experimental system at the time when phase-conjugate light is generated. From the phase distribution calculated in the previous process, a phase-conjugate pattern for P-SLM is calculated. The P-SLM is irradiated with the intensity-modulated reference light. The spatially modulated light is reflected by the phase-conjugate pattern. The phase-conjugate light generated in this manner propagates back through the path of the signal light and again passes through the scattering medium. This transmitted light is recorded by another CMOS camera (CMOS camera 2, DCC 1545 M; Thorlabs, Inc.). If both the phase and intensity of phase-conjugate light are correct, then the shape of the signal light before incidence at the scatterer is expected to be recorded on CMOS camera 2 because of its time-reversal property. For this study, we conducted experiments under the three additional conditions (PM, IM, NoM) for comparison along with the above condition, or phase and intensity modulation (PIM). First, we did not assign an intensity distribution to I-SLM making the uniform intensity and the modulated phase distributions (PM). This condition is the same as that of the conventional method. Second, the phase distribution was not assigned to the P-SLM, which made the modulated intensity and the uniform phase distributions (IM). Finally, neither I-SLM nor P-SLM was assigned a spatial distribution. No modulation was made on the reflected light (NoM). This is a condition of simple transillumination imaging without scattering suppression.

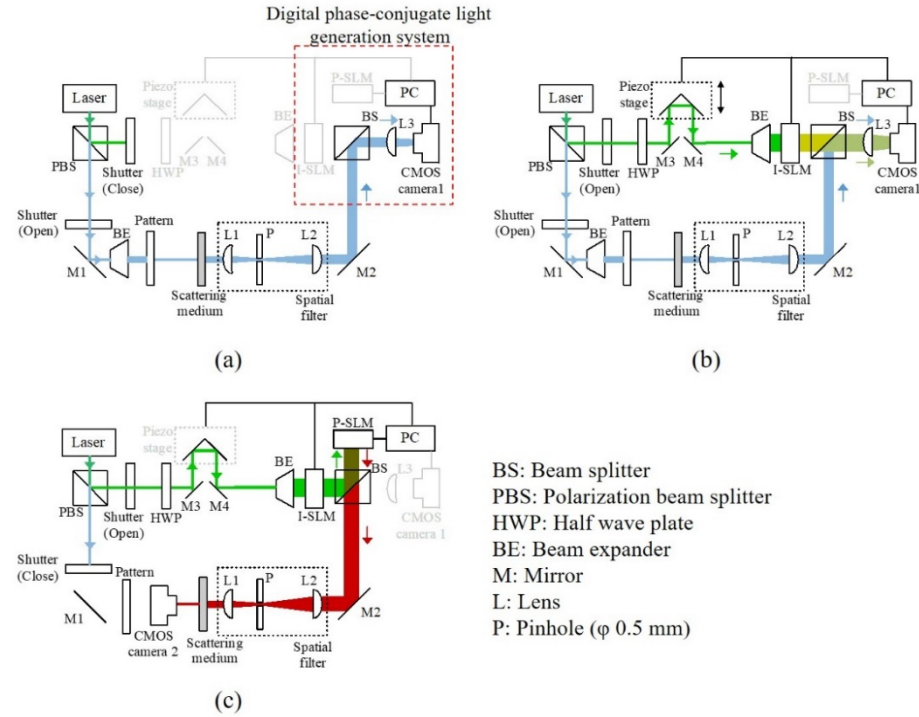


Fig. 3. Experimental system to restore the incident light shape from scattered light: (a) intensity recording, (b) phase recording, and (c) generation of intensity and phase modulated light.

4. Restoration of incident light shape with artificial scattering medium

To confirm the basic performance of the developed system, we conducted an experiment to restore a simple cross section of an incident Gaussian beam before scattering from the light obtained after scattering. As a scattering medium, an artificial medium (polypropylene sheet, thickness 2 mm, reduced scattering coefficient $\mu_s' = 0.004$ /mm) was used. This weakly scattering material was easily controlled. Its structure, which was more homogeneous than that of the animal sample, increased the reliability of confirmation.

The experimentally obtained results are portrayed in Fig. 4. Figure 4(a) portrays the shape of the incident light before the scatterer. This image corresponds to the original image without the blur caused by scattering. Figure 4(b) presents the light after passage through the scatterer captured with CMOS camera 2, under the condition of no modulation (NoM). However, Fig. 4(c) presents a recording made by CMOS camera 2 under the condition with phase-conjugate and intensity modulation (PIM). The intensity profiles in the horizontal direction of the center of each image are depicted in Figs. 4(d)–4(f). The conjugate phase pattern used for phase modulation and the intensity pattern used for intensity modulation are shown in Fig. 4(g) and Fig. 4(h). In the case of simple transmission, light was diffused considerably by scattering (Fig. 4(b)). In the condition of intensity and phase modulation (Fig. 4(c)), the image was restored to approach the original shape of the incident light according to the time-reversal principle.

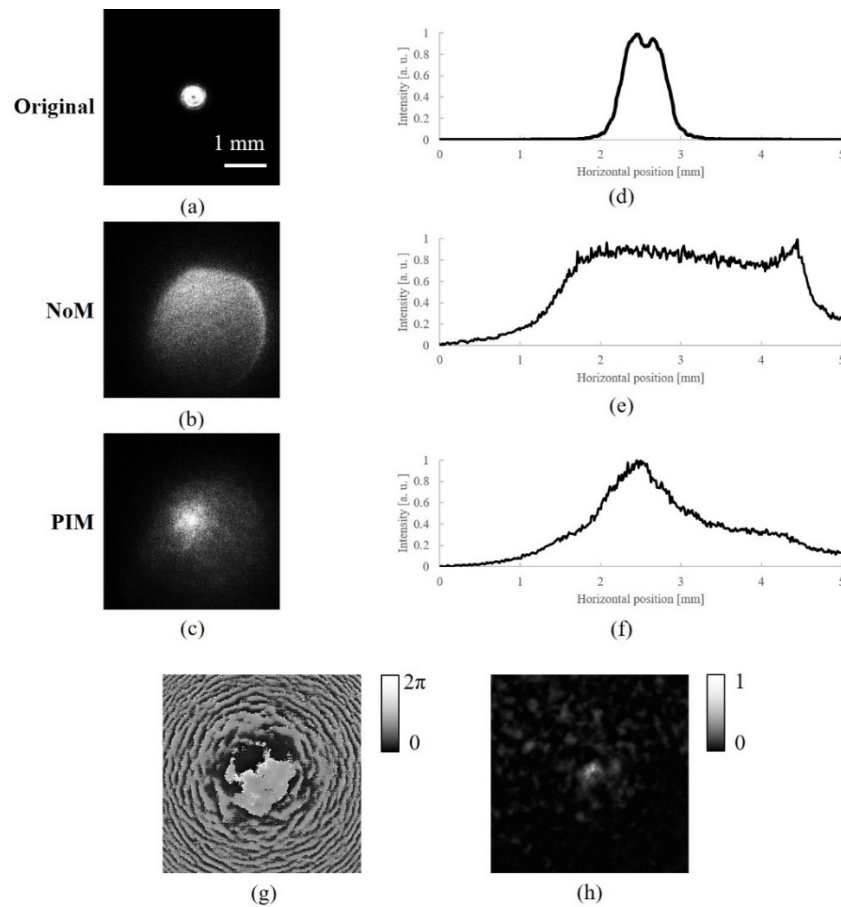


Fig. 4. Verification for generation of phase-conjugate light: (a) incident light pattern, (b) observed image with no restoration (NoM, SBR = 1.25, CC = 0.27), (c) image with phase-conjugate and intensity modulation (PIM, SBR = 2.09, CC = 0.55), (d)–(f) horizontal profiles at the center of (a)–(c), (g) conjugate phase pattern of P-SLM, and (h) intensity pattern of I-SLM.

To evaluate the quality of the restored image, the signal-to-background ratio (SBR) and the correlation coefficient (CC) were calculated as shown in the figure caption. From these results, we confirmed that the obtained image was the result of the time reversal effect of the phase-conjugate light, and that it was not a burn-in trace of the imaging device or an artifact of the experimental system, such as a lens-focusing effect.

We conducted a restoration experiment of a known signal pattern. As the signal image, a transmitting USAF pattern was used. A selected area of 1.78 /mm (0.56 mm line width) was imaged. Experimentally obtained results are presented in Fig. 5.

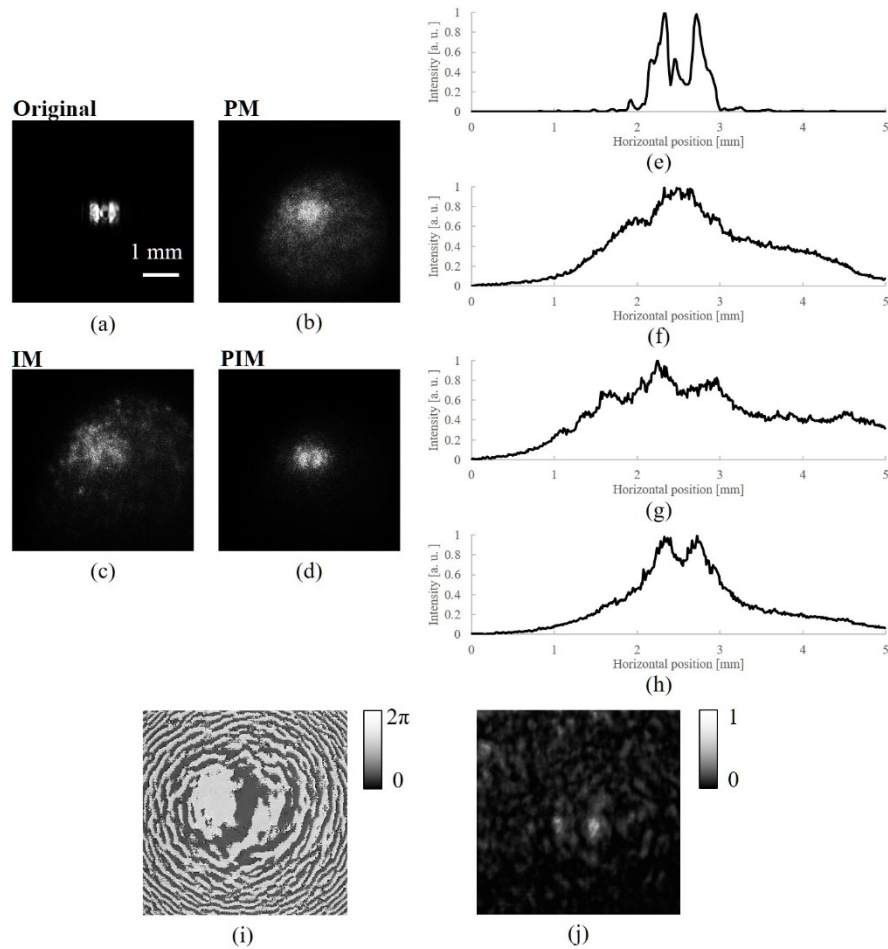


Fig. 5. Contribution of intensity and phase components on scattering suppression using phase-conjugate light: (a) incident light pattern, (b) observed image with phase-conjugate modulation (PM, SBR = 1.62, CC = 0.33), (c) observed image with intensity modulation (IM, SBR = 1.86, CC = 0.46), (d) image with phase-conjugate and intensity modulation (PIM, SBR = 2.72, CC = 0.65), (e)–(h) horizontal profiles at the center of (a)–(d), (i) conjugate phase pattern of P-SLM, and (j) intensity pattern of I-SLM.

Figure 5(a) is an image of the intensity distribution of the irradiated light observed at the light-incident side of the scatterer. Figures 5(b)–5(d) are images taken respectively in conditions of PM, IM, and PIM through time-reversal imaging. Intensity profiles in the horizontal direction of the center of each image are shown, respectively, in Figs. 5(e)–5(g). The conjugate phase pattern used for phase modulation and the intensity pattern used for intensity modulation are presented in Fig. 5(i) and Fig. 5(j). These comparisons confirmed that the blur caused by scattering is suppressed by the phase-conjugate light. Furthermore, the results clarified that the addition of intensity information to the phase modulation provides a synergistic effect rather than additive effect for improving the image restored using the time-reversal principle.

5. Characteristic analysis of the proposed method

Results show that the blur caused by scattering can be suppressed effectively by adding intensity modulation to the phase-conjugate light. To clarify this mechanism, the incident light shape was restored while changing the reduced scattering coefficient of the scatterer

using the system depicted in Fig. 3. A similar study was conducted using the light focusing system [16]. In the present study, we examined it with non-focusing system to analyze the characteristics of the phase-conjugate light itself.

In the experiment, a mixture of agar and intralipid suspension (Fresenius Kabi AG) as a scatterer was enclosed in an optical cell having 3 mm thickness and was then solidified. The reduced scattering coefficient was varied in the range of $\mu_s' = 0.23\text{--}0.40$ /mm by adjusting the intralipid suspension solution concentration. In this study, the incident light pattern was generated using a pattern of line pair 1.00 /mm (1.00 mm line width) of the USAF target.

The experiment results are depicted in Fig. 6. Figure 6(a) depicts the intensity distribution of the light incident on the scatterer when the experimental system was in the intensity recording mode (Fig. 3(a)). Figures 6(b)–6(e) are images taken with phase information only (PM). Figures 6(f)–6(j) are images taken when intensity information was added (PIM). The reduced scattering coefficient (μ_s'), optical distance (OD; cell thickness multiplied by μ_s'), and signal-to-background ratios (SBR) and the correlation coefficients (CC) with respect to Fig. 6(a) are also presented in the figure.

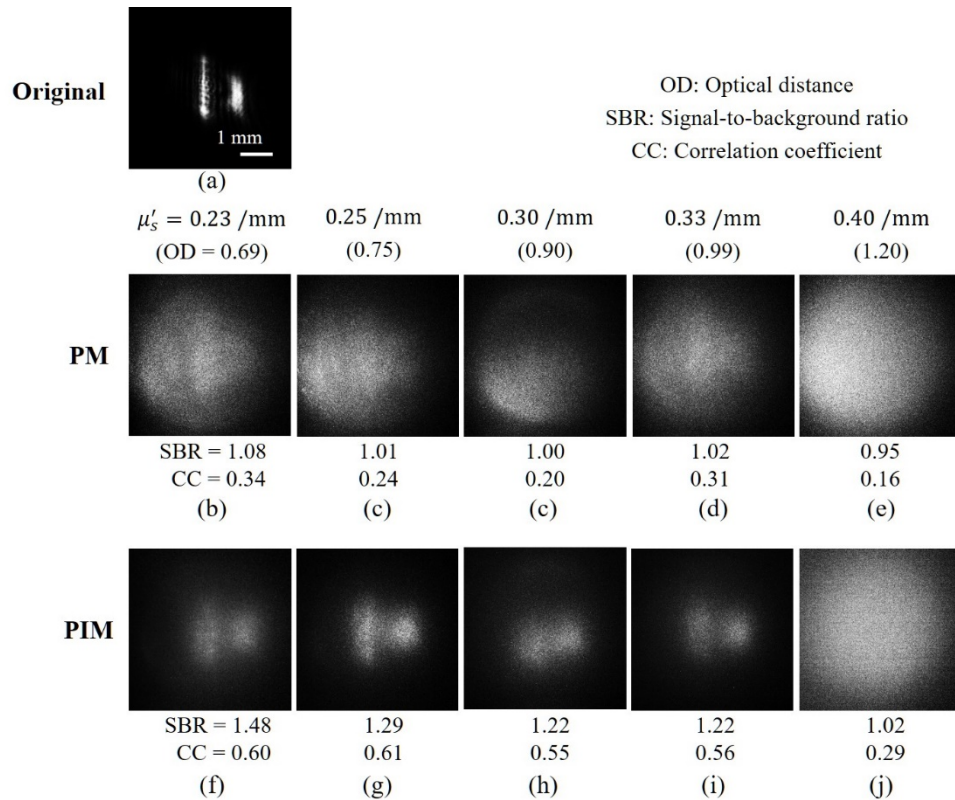


Fig. 6. Dependence of restored image on reduced scattering coefficient of a turbid medium: (a) incident light pattern, (b)–(e) observed images with phase-conjugate and no intensity modulation (PM), and (f)–(j) observed images with phase-conjugate and intensity modulation (PIM).

When only phase information is used, the pattern of the original image is observed slightly at $\mu_s' = 0.23$ /mm. With a greater reduced scattering coefficient, it is difficult to recognize the pattern, but when intensity modulation is added, the blurring caused by scattering is effectively suppressed for $\mu_s' = 0.23\text{--}0.33$ /mm. When $\mu_s' = 0.40$ /mm, the pattern cannot be recognized. From this analysis, OD = 1 is regarded as the threshold boundary. These results demonstrated that the scattering suppression capability of this system is as

effective for scattering medium with $OD < 1$ as it is for the characteristic of phase-conjugate light with non-focusing system.

6. Restoration of incident light shape with an animal sample

To assess the applicability of the proposed technique to inhomogeneous animal tissues, a similar experiment was conducted using an animal sample as the scatterer. The experimental system depicted in Fig. 3 was used for this experiment. For the target image, a pattern of line pair 1.4 /mm (0.71 mm line width) of the USAF target was used.

Preparation of the animal sample used as the scatterer is portrayed in Fig. 7. To maintain the sample thickness constant, we used a microscope coverslip (0.15 mm thickness) as a spacer and sandwiched a sample of minced chicken breast meat between microscope slides. Although the reduced scattering coefficient of the chicken breast meat is $\mu_s' = 0.50$ /mm at 532 nm wavelength [22]. As might be apparent in Fig. 7(c), even at this thickness, the sample shows strong scattering and an optically inhomogeneous structure.

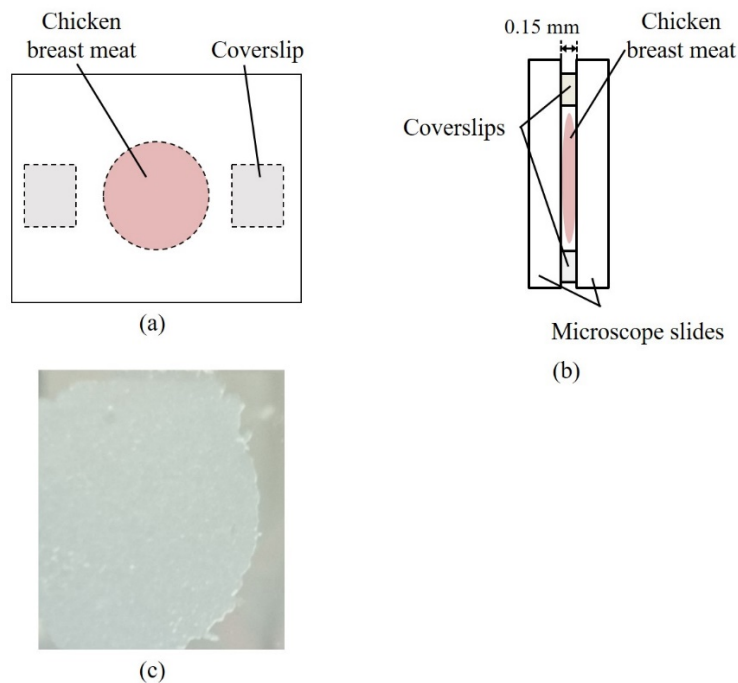


Fig. 7. Animal tissue sample (chicken breast meat): (a) front view of sample structure, (b) side view of sample structure, and (c) appearance.

Results of the experiment are presented in Fig. 8. Figure 8(a), which depicts an image of the incident light pattern irradiated at the scatterer, corresponds to the original image. Figures 8(b) and 8(c) respectively present images of conditions PM and PIM in time-reversal imaging. Figures 8(d)–8(f) depict intensity profiles in the horizontal direction of the central part. As shown at the center of Fig. 8(b), only an unclear light pattern was restored using the conventional method. However, an image depicting the structure of the original pattern was reproduced when the intensity information was added, as in Fig. 8(c).

In addition, a noise suppression effect is visible around the restored incident pattern. The cause of this effect is regarded as follows. In image restoration with conventional phase-conjugate light alone, noise is emphasized in areas where the signal light intensity is low because the phase-conjugate light is generated from the reference light with uniform intensity distribution. However, when intensity information is added, the intensity distribution

suppressed the signals in such region. This experimentally obtained result demonstrates that the principle of time-reversal by phase-conjugate light with intensity information is effective for suppressing the scattering effect in inhomogeneous animal samples.

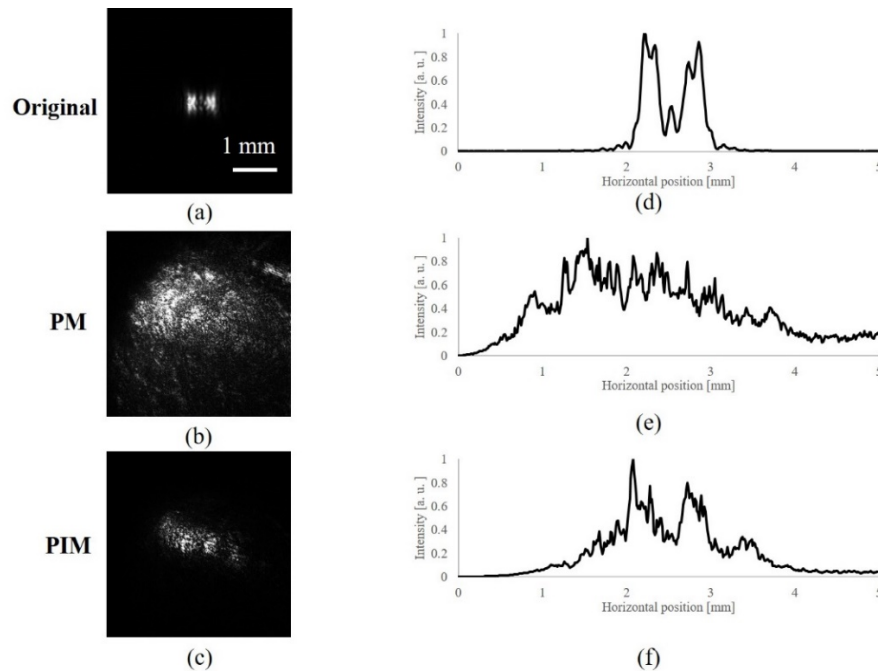


Fig. 8. Verification for the influence of the intensity component on an animal sample: (a) incident light pattern, (b) observed image with phase-conjugate modulation (PM, SBR = 1.31, CC = 0.18), (c) image with phase-conjugate and intensity modulation (PIM, SBR = 3.28, CC = 0.51), (d)–(f) horizontal profiles at the center of (a)–(c).

The chicken breast meat used for this experiment was extremely thin: 0.15 mm thickness. The reason for this thickness derives from the practical limit of our experimental system. If scattering suppression is effective through a scattering medium with $OD = 1$, then we can restore the image through 2-mm-thick chicken breast meat with $\mu_s' = 0.50 / \text{mm}$ [22]. Therefore, we can expect to increase the sample thickness further by improving the experimental system performance. Another factor that might accommodate increased sample thickness is the coherent length of a light source. Wang et al. focused phase-conjugate light through 25-mm-thick chicken breast [16]. This difference in capabilities apparently derives from the coherence length of the light source. The coherence length necessary for interference through a scatterer is proportional to the square of the scatterer thickness [16]. The coherence length of the light source used in this experiment was approximately 10 mm, which is only one ten-thousandth of the length reported from an earlier study by Wang et al. (coherence length 100 m or more, Verdi V10; Coherent Inc.). Therefore, theoretically, if using a light source with 10^4 times greater coherence length in our system, we can increase the scatterer thickness by 10^2 times. As a result, we can expect to restore the image through chicken breast meat of 10–15 mm thickness using our simple optical system. For this study, we strove to produce a simple imaging system to clarify the intensity information effect. Therefore, some room for improvement of the imaging performance of this system remains, perhaps by adding a polarizer in front of a camera or using a light source for providing light with longer coherent length. Optimization of such experimental systems remains as a subject for future work.

For this experiment, measurements of the phase information of the signal light and generation of phase-conjugate light were all performed offline. During this process, the

scattering medium was fixed. However, it is not easy to satisfy this requirement for *in vivo* applications. In the case of dynamic scatterers such as an animal tissue, the time-reversal effect of phase-conjugate light is degraded considerably [23]. Some basic techniques are expected to be useful to resolve this difficulty [24–27]. They include one which captures the intensity distribution and the phase distribution of signal light using a one-shot laser irradiation [24], and one which realizes high-speed phase-conjugate modulation using digital micromirror devices [27]. By introducing these techniques, the proposed technique can be an effective means of suppressing the scattering effect, even for *in vivo* applications.

7. Conclusion

With a view toward the suppression of scattering effect in optical transillumination imaging of animal tissues, we examined the effect of intensity modulation for the time-reversal function of digital phase-conjugate light. First, a digital phase-conjugate light generation system capable of simultaneous modulation of intensity and phase distributions was constructed with transmission and reflection SLMs. Using this system, we attempted to restore the incident light pattern before scattering from the light after passage through the scatterer. Results clarified that, compared to the conventional use of phase modulation alone, the restoration performance is improved remarkably by adding intensity modulation. Next, dependence of the restoration performance on a reduced scattering coefficient was investigated. Consequently, we confirmed that the scattering effect can be suppressed in the range of $OD \approx 1$ or lower. This is the characteristic of the phase-conjugate light in transillumination imaging with non-focused light. Finally, we attempted to restore the incident light pattern using chicken breast meat as a scattering medium. Results demonstrated that the time-reversal ability was improved and that the scattering effect can be suppressed by adding intensity information to the phase-conjugate light, even for animal samples having an inhomogeneous structure.

This study demonstrated that we can expect to suppress the scattering effect using the time-reversal principle without using a confocal system. Moreover, it was demonstrated that the addition of intensity modulation to the phase-conjugate light apparently improves the image blurred by the scattering effect. Applicability of the proposed technique to the inhomogeneous animal scattering medium was demonstrated in experiments.

To extend the usefulness of the proposed technique, important limitations of the current experimental system must be resolved. They include the increase of the light source coherent length and the reduction of the measurement time while maintaining the scattering medium stability.

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Disclosure

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